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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

HIBINO

Attorney Docket No. 100021-09042

Application Serial No. 09/444,388

Group Art Unit: 1655

Filing Date: November 22, 1999

Examiner: J. Souaya

For: PROCESS FOR OBTAINING PLANT DNA FRAGMENTS AND USE THEREOF

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

June 5, 2002

Sir:

This Preliminary Amendment is being filed in response to the final Office Action of December 5, 2001 for the above application, along with a three-month Petition for Extension of Time and the statutory fee, making the response due on or before June 5, 2002.

Please amend the application as follows.

IN THE CLAIMS:

Please cancel claims 9 and 16 without prejudice or disclaimer.

Please amend claim 8 as follows. A marked-up copy of the claim is included as an attachment pursuant to 37 C.F.R. §1.121.

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8. (Amended) A method for obtaining a DNA fragment for a breeding marker for polymorphic forest tree plants, comprising the steps of:

C1

a) selecting two sibling individuals of a plant having different phenotypes;

b) obtaining genomic DNA from the two individuals;

c) selecting DNA fragments by an inter-individual genome subtraction method using the genomic DNA from the two individuals;

d) providing an RNA-derived labeled probe, wherein the probe is a labeled cDNA of all mRNA obtained from the two individuals, and the cDNA is selected and amplified by oligonucleotide primers in a polymerase chain reaction, wherein the primers are designed to hybridize to the mRNA for a plant gene related to the breeding marker;

e) fractionating the DNA fragments obtained by the genome subtraction of step c) and screening the DNA fragments with the RNA-derived labeled probe of step d);

f) repeating steps c) to e) with genomic DNA from one of the two individuals; and

h) comparing the DNA fragments of steps e) and f) to exclude the DNA fragments containing intra-individual polymorphisms and to identify the DNA fragment for the breeding marker.

10. (Amended) The method of claim 8, wherein the forest tree is Acaia.

11. (Amended) The method of claim 10, wherein the Acacia is a species *Acacia auriculiformis*.

REMARKS

The final Office Action of December 5, 2001 has been received and carefully noted, and the foregoing amended claims and the following comments are a complete response thereto.

Claims 8-16 are all the pending claims, and by this Amendment, Claims 8, 9 and 16 have been combined to recite a method for obtaining a DNA fragment for a breeding marker in polymorphic forest trees. The amendment made to Claim 11 is cosmetic and no change in scope has been introduced. Claims 9 and 16 are canceled without prejudice or

disclaimer. No new matter has been added, and consideration and entry of the amended claims is requested.

I. Response to Rejection of Claims 8-16 under 35 U.S.C. §112, first paragraph

Claims 8-16 are rejected under 35 U.S.C. §112, first paragraph, for lack of written description support.

In the Office Action, the Examiner alleges that the claims are broadly drawn to a process for obtaining any plant DNA fragment by digesting DNA from any plant, subjecting the fragments to subtractive hybridization to obtain polymorphic fragments and screening the fragments to obtain a desired fragment. However, the specification only teaches a general method of obtaining DNA fragments from plants using genome subtraction, and does not teach whether these fragments contain "breeding markers" as encompassed by the claimed invention. Thus, the Examiner considers the claimed subject matter as lacking in written description support.

Applicants traverse the Examiner's rejection for the following reasons.

Regarding the scope of the plants to which the claimed invention is now directed, Applicants submit that the specification provides written description support for "forest trees" (page 1, line 29; or "arboreous plants" including more specifically, poplar, eucalyptus and acacia trees (page 8, lines 14-15)). Accordingly, one skilled in the art can look to the original specification for the scope of forest trees to which the instant claimed method can be applied in identifying breeder markers for both inter-individual and intra-individual comparison of polymorphic traits.

Applicants submit that the claims meet the statutory requirements under the first paragraph of 35 U.S.C. §112 and that withdrawal of this rejection is deemed proper.

II. Response to Rejection of Claim 11 under 35 U.S.C. §112, second paragraph

Claim 11 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite.

According to the Examiner, Claim 11 is indefinite for the recitation of "Acacia auricaliformis" since the only known species in the art is "Acacia auriculiformis".

The foregoing amendment to claim 11 is only cosmetic, and the amendment does not change the scope of the claim. Withdrawal of the Examiner's rejection is deemed proper.

III. Response to Rejection of Claims 8, 15 and 16 under 35 U.S.C. §102(b)

Claims 8, 15 and 16 are rejected under 35 U.S.C. 102(b) as anticipated by Phillips (Plant Molecular Biol. 24:603-615 (1994)).

According to the Examiner, the claims are presently written to encompass prior art such as Phillips for reasons of record, and the claims read on the reference.

Applicants submit that Phillips does not teach or suggest all of the claimed elements and traverse the Examiner's rejection of the claims for the following reasons.

Forest trees are characterized in that they exhibit intra-individual heterogeneity on a genomic level. In other words, for any one individual forest tree, one observes individual DNA heterogeneity within a given tree. This property can influence the interpretation of any genomic characterization of an individual plant much less any comparison made between different plants of the same species. Therefore, after genomic subtraction of DNA obtained from two different individual trees of the same species, the resultant DNA represents not only differences in DNA between the two individuals, but differences at the DNA level that are noted on an individual basis, i.e., intra-individual heterogeneity. Thus, in order to make a more valid genomic comparison between two different individuals, DNA heterogeneity

occurring within any one of the two individuals should be subtracted from the genomic profile.

Phillips only teaches an inter-individual **cDNA/mRNA** subtraction method (see section entitled "Construction of enriched cDNA samples" and Figure 1 on page 605) for use in characterizing gibberellin-induced mRNA expression in Arabidopsis (cereal grains) compared to untreated controls. The reference is silent with respect to an inter-individual **genomic DNA** subtraction method step according to step c) of Claim 8.

Still further, Phillips does not teach the occurrence of intra-individual genomic DNA heterogeneity in Arabidopsis cereal plants much less forest trees. Phillips does not appreciate this aspect of the substrate DNA for this invention, and the reference disclosed method omits yet another essential step of the present invention (i.e., step f) of claim 8), namely, the subtraction of the genomic DNA from a given plant against its very own genomic DNA in order to eliminate intra-individual heterogeneity.

For the present invention, the subtraction of genomic DNA between individual forest trees is performed according to steps a) to e) of Claim 8. Step f) of Claim 8 represents the step where individual DNA heterogeneity is eliminated by subtracting a given forest tree's genomic DNA with its own genomic DNA. Phillips does not teach either of these steps in its disclosure. Step h) of Claim 8 is where the steps of e) and f) are compared in order to more precisely identify DNA fragments containing a given breeder marker and any differences that may occur between individual forest trees.

Phillips teaches identifying novel cDNAs, the expression of which, are induced upon exposure to gibberellin. The present invention is a method for obtaining a DNA fragment usable as a breeding marker, and is not directed to identifying a novel DNA marker. The

primers for the inventive method are designed to hybridize to the mRNA for a plant gene related to a breeding marker.

With respect to instant claim 15, Phillips does not teach fractionating a subtracted **genomic DNA fragment** on polyacrylamide gels. Phillips teaches fractionating **cDNAs or mRNAs** on polyacrylamide gels. Claim 16 has been canceled, thereby rendering the Examiner's rejection moot with respect to this claim.

The Examiner has not established a *prima facia* case of anticipation for Claims 8 or 15, and therefore the Examiner's rejection of these claims is not sustainable in view of Phillips.

IV. Response to Rejection of claims 9-11 under 35 U.S.C. §103(a)

Claims 9-11 are rejected under 35 U.S.C. §103(a) for being obvious over Phillips in view of Pinyopusarerk (ACIAR proceedings, 1987, no. 16, pp 147-148).

The Examiner considers claims 9-11 *prima facia* obvious in view of Phillips and Pinyopusarerk, since Phillips teaches a genomic subtraction method for the identification of breeder marker genes with a specific phenotype, and Pinyopusarerk teaches a need for improving the quality of plant forest trees, specifically Acacia auriculiformis stem axis formation, by selection and breeding.

Applicants traverse the Examiner's rejection of Claims 9-11 in view of Phillips and Pinyopusarerk for the following reasons.

Phillips teaches a cDNA/mRNA subtraction strategy for identifying novel gene expression in Arabidopsis. One skilled in the art would not even consider the method of Phillips as approximating the genomic subtraction strategy of the instant claims. Each of the methods relies on different substrate materials and the order of steps for obtaining the

readout for the respective methods. Phillips method is designed to identify novel gene expression under conditions where gene expression is specifically induced compared to a resting control. The inventive method of claim 10 and 11 is designed to identify and compare genomic DNA fragments containing genes for breeder markers expressed among individual forest tree plants for Acacia. Thus, because Phillips' overall method cannot even be considered to fall within the same field of art as the inventive method of Claim 8, the Examiner has not established a *prima facia* case of obviousness for Claims 10-11 (Claim 9 having been canceled renders the Examiner's rejection moot with respect to this claim).

Since Phillips' method completely departs from the nature and scope of the present invention, the Examiner has not met his burden in establishing why one skilled in the art would have been motivated to rely on the limited disclosure of Pinyopusarerk to rectify any of these deficiencies. Significantly, Pinyopusarerk is silent with respect to any molecular biological methodologies that can be used in selecting and breeding Acacia auriculiformis much less any other form of plant. Pinyopusarerk is insufficient in its disclosure with respect to teaching any detailed method steps; the reference only provides a generic teaching of the field and one skilled in the art would not have been motivated to combine the reference with Phillips. Applicants further submit that one skilled in the art would not even have had a reasonable expectation of success in combining the references. For all of the foregoing reasons, Applicants submit that the Examiner's obviousness rejection is not sustainable.

V. Response to Rejection of claims 12-14 under 35 U.S.C. §103(a)

Claims 12-14 are rejected under 35 U.S.C. §103(a) for being obvious over Phillips in view of Wigler and Nainan (J.Virol.Methods, 1996, 61:127-134).

The Examiner considers the claims *prima facia* obvious over Phillips, Wigler and Nainan, since according to the Examiner, it would have been obvious to use the RDA method of Wigler in the genomic subtraction method of Phillips for obtaining a more sensitive comparison of individual genomes. Further, the Examiner considers combining the teachings of Wigler and Phillips with Nainan obvious, since Nainan teaches labeling cDNAs with specific labels for increasing sensitivity of cDNA detection.

For purposes of brevity, Applicants incorporate all of the arguments for patentability of the claims over Phillips as previously discussed.

With respect to Wigler, it is entirely unapparent why the Examiner considers the reference disclosure as rectifying Phillips deficiencies, when each of the subtraction methodologies is technically distinct. Again, Phillips teaches a cDNA/mRNA subtraction technique for identifying novel expressed cDNAs in plants. On the other hand, Wigler teaches representational difference analysis (RDA) which relies on subtracting genomic DNA from two different individuals of a like organism (such as plants) to identify genetic polymorphisms.

Claim 12 is directed to RDA, but there is no motivation provided by either one of the references alone or in combination, as to how one should combine their disclosures to obtain the overall method of Claim 8 or even a method of claim 8 more specifically directed to a step using RDA. The inventive method is directed to two DNA genomic subtraction steps; the first for subtracting genomic DNA fragments between two individuals of a given species, followed by the second step, where the selected DNA fragments from the one individual of the first step are subtracted against its own genomic DNA to reduce background noise.

Neither Phillips nor Wigler teaches or suggests that intra-individual heterogeneity occurs within a given individual, or that this genomic heterogeneity can contribute to what is considered background interference when making an inter-individual comparison at a molecular level. The method of Claims 8 and 12 are unique and nonobvious for this essential feature, namely, for removing or excluding individual genomic heterogeneity and providing an improvement over the methods in the Examiner's cited references, and more particularly, Wigler.

Naiman is cited by the Examiner for teaching labled cDNAs. Labeled cDNAs are used in a step of the present invention (Claims 8 and 13), but other essential elements of the invention are not taught or suggested by either Naiman with Phillips or Wigler.

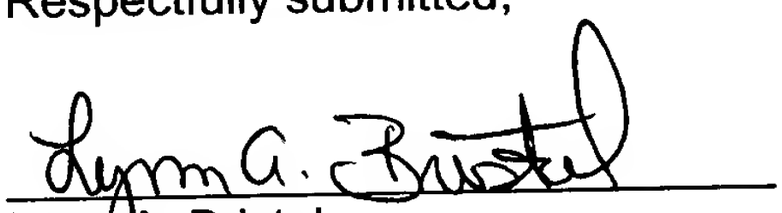
Applicants submit that the methods described in the Examiner's cited references are so disparate in there manner of performance and resultant outcomes, that the present genomic subtraction method is nonobvious over these references alone and in combination.

CONCLUSION

Applicants have demonstrated that each of the Examiners cited references teach distinct and separate methods from not only each other, but in view of the instant claimed method. Accordingly, based on these arguments and the amended claims, the Examiner's rejections of the claims under 35 U.S.C. §§102(b), 103(a) and 112, second paragraph, have been met and overcome. Applicants now request that the Examiner allow this application to pass to issuance.

In the event that any fees are due in connection with this paper, please charge our Deposit Account No. 01-2300 referencing Docket No.100021-09042.

Respectfully submitted,



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